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ORIGINAL ARTICLE

The feasibility of sentinel lymph node biopsy for gastric cancer: the experience from Serbia

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Summary

Purpose: The prediction of outcome for patients with gastric cancer is determined largely by the presence of lymph node metastases, which could be detected by sentinel lymph node (SLN) biopsy (SLNB). The purpose of this work was to determine the feasibility of SLNB in patients with gastric cancer for the assessment of regional lymph node status, including performing immunohistochemical (IHC) staining of SLN tissue.

Methods: We reviewed 137 consecutive patients with operable gastric cancer over a 10-year period using a retrospective (to examine skip metastases) and prospective (to evaluate successful mapping) study design. SLNs were mapped, biopsied and subsequently explored by routine hematoxylin & eosin (H&E) staining and by IHC staining using a cytokeratin 8/18 antibody.

Results: The retrospective study showed a low incidence of skip metastases (3.7%). Mapping of SLNs in the prospective study was highly successful (98.2%). During the prospective study, IHC examination of SLNs from 56 patients showed statistically significant change in disease stage.

Conclusion: This study demonstrated highly successful mapping and biopsy of SLNs (98.2%), as well as highest specificity (100%), sensitivity (100%) and accuracy (100%) to predict metastasis in the surrounding lymph nodes of gastric carcinoma. In addition, we believe that IHC study might enable "ultra staging" and additional selection of patients for further cancer treatment.

Key words: biopsy, gastric cancer, immunohistochemistry, sentinel lymph nodes

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Introduction

Gastric cancer is the second leading cause of cancer-related mortality worldwide [1]. The prognosis of patients with gastric cancer is determined mostly by the occurrence of lymph node metastases [2]. SLN is characterized as the first lymph node which receives lymphatic drainage from the primary tumor [3]. The concept of examining SLNs is based on the hypothesis that a metastasis in SLN could indicate metastases in other lymph nodes, whereas a negative SLN indicates that the rest of the lymph nodes are tumor-free.

SLNB was first performed in penile cancer, and the technique has been widely used in melanoma and breast cancer [4-6]. In 1992, Morton et al. [5] introduced the technique of intraoperative dye injection at the site of melanoma to identify the "sentinel" node, which is the first node that the afferent lymphatics enter from the tumor site. SLNB for gastric cancer is an intraoperative diagnostic method to detect lymph node metastases [7] and SLN mapping in patients with gastric cancer has been performed in the past 10 years [8-11]. Intraoperative detection of SLN metastasis during gastric cancer surgery is the key factor that will determine whether a patient will proceed with conventional D2 lymph node dissection or not [12]. Most of the studies in this field have been conducted in Japan, where adenocarcinoma of the stomach is considered endemic and remains the leading cause of cancer-related deaths [13].

In routine practice, SLNB in patients with gastric carcinoma is based on the use of H&E staining for intraoperative histological examination of frozen section material. However, the success of this intraoperative method is controversial, since Kitagawa et al. [12] reported that accurate intraoperative diagnosis using a single H&E-stained section was possible in only 74% of all cases. On the other hand, other authors reported satisfactory accuracy rates using H&E staining of SLNB between 93.8 and 100% [14].

There are several different studies in which the conventional H&E method was compared with more sophisticated methods, such as IHC staining [14]. These studies reported a considerable improvement in the detection rate where the presence/absence of metastasis was confirmed using IHC. However, a recent meta-analysis indicates that SLNB in gastric cancer is technically feasible with an acceptable sensitivity and suggests that further research is needed to confirm the best procedure and standard criteria [15].

The aim of our research was to determine the feasibility of SLNB in patients with gastric cancer for the assessment of the regional lymph node status, including IHC studies.

Table 1. Patient inclusion and/or exclusion criteria

Patient groups	*Retrospective study (N=81)*	*Prospective study (N=56)*
Inclusion criteria	Solitary invasive tumor confined to one part of the stomach	Patient older than 18 years
	Curative gastrectomy with D2 or D3 lymphadenectomy	Palpable tumor at the time of the surgery
	Histologically determined presence of metastases in only 1-2 lymph nodes	Histologically confirmed primary carcinoma of the stomach
Exclusion criteria		Previous irradiation or chemotherapy.
		Non-palpable tumor at the time of surgery.
		Recurrent gastric cancer.
		Distant metastases.

Methods

Patients

We reviewed 137 consecutive patients with operable gastric cancer, without metastases or peritoneal dissemination over a 10-year period (January 1999 to January 2009). All of the patients were operated on in the Department of Surgery, University Hospital "Dragisa Misovic" in Belgrade, Serbia. A combined retrospective/prospective study design was employed.

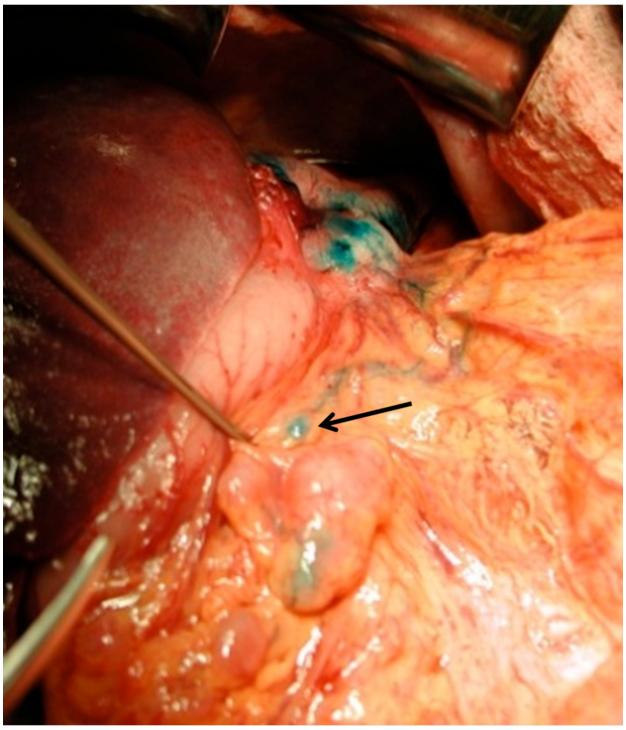

Figure 1. The first lymph node colored by injected vital dye is marked as the sentinel lymph node (arrow).

In 56 patients that were analyzed in the prospective study from January 2005 to January 2009, intraoperative SLNB was performed. Before performing SLNB, written informed consent was obtained from patients in accordance with the standards of the Ethical Committee of the Medical School, University of Belgrade. The data collected in the prospective study included age, gender, tumor location, resection type, tumor stage and histopathological characteristics (macroscopic type, histological type, Lauren's and UICC classification, tumor size and depth invasion, status of SLN and all lymph nodes).

For the retrospective study, we retrospectively analyzed the medical records from 81 patients who had undergone surgery for gastric cancer from January 1999 to January 2005 and had histopathologically identified metastases in only 1-2 lymph nodes. The inclusion and exclusion criteria of patients into the study are summarized in Table 1.

Procedure for detection of sentinel lymph nodes

During the operation the abdominal cavity was explored in 56 patients with gastric cancer, and disease stage and the feasibility of resection were assessed. In order to map SLNs, the previously described standard technique of intraoperative subserosal injection of dye was applied [10,16,17]. A tuberculin syringe and needle were used to inject 1ml of vital dye Isosulfan blue (1% Lymphazurin aqueous solution; Ben Venue Labs, Bedford, OH) subserosally at 4 different opposing points around the gastric tumor. SLNs were defined as the first-appearing colored lymph nodes, 5 min after injection of the tracer (Figure 1). Subsequently, SLNs were carefully excised and sent for histopathological examination.

The SLNs were cut along the plane of the largest diameter that included the node hilum, and frozen sections were stained with H&E and examined intraoperatively for metastases. Patients without established lymph node metastasis underwent routine D2 lymphadenectomy as standard procedure [12]. Afterwards, other regional lymph node groups were removed from the stomach. All lymph nodes were grouped according to the Japanese classification of gastric carcinoma [18].

Immunohistochemistry

Resected lymph nodes specimens and the remaining frozen tissues from the SLN were routinely cut at 0.2 cm sections, fixed in 10% formalin, processed and embedded in paraffin for permanent storage. Serial 3-μm thickness sections were cut from each lymph node. The first section was H&E-stained and the second section was placed on a Superfrost Plus Slide for further staining. The H&E-stained slides were examined by a pathologist and if there was no evidence of metastatic involvement, the consecutive slides were stained with cytokeratin 8/18 antibody (NCL-C50, Novocastra, UK). For the qualitative identification of antigens we used the Envision system (DAKO, Carpinteria, CA).

Statistics

Statistical analyses were performed using SPSS software for Windows ver. 12.0 (SPSS Inc., Chicago, IL, USA). P-values of <0.05 were regarded as statistically significant. Continuous data were presented as means

± standard deviations, and were analyzed using the Student's t-test. The chi-square test was used to analyze categorical data.

Results

In the retrospective study 81 patients (males n=53; 65.4% and females n=38; 34.6%) with average age 65±6.47 years and with 1-2 metastatic lymph nodes were chosen for further investigation. These patients were considered as patients with possible metastases in SLNs, and their distribution was analyzed to determine the frequency of skip metastases. Patients with N2 and N3 nodes without N1 metastases were considered to be patients with skip metastases. The characteristics of the 81 patients included in this study are summarized in Table 2. As shown there, only 3 patients showed N2 nodal involvement without N1 nodes, which defined them as skip metastases. Skip metastases were detected in patients with central and proximal location of gastric tumors (Table 2). Further analysis showed a correlation between the presence of skip metastases and T3 and T4 tumors ($p<0.001$) (Table 2). Skip metastases were detected in patients with infiltrative and signet ring cell carcinoma types.

In the prospective study, 56 patients had SLNB. Demographic and clinical characteristics of these patients are shown in Table 3. Out of 56 patients 60.3% were males and 39.7 % females with average age 63. 91±9. 37 years; most of them were operated on within 3-6 months of diagnosis (Table 3). *Helicobacter pylori* was identified in 61.7% of all patients. Most patients suffered from pain and dyspepsia, weight loss and vomiting, while a minority had bleeding and gastric ulcer (Table 3). Tumor was detected mainly in the distal part of the stomach (48.2%), with the remainder occurring in the proximal and central parts of the stomach. In about 1/3 of all patients, the tumor was less than 2.75 cm^3 (Table 3). According to Bormann's classification system of tumors, 51.8% of operated gastric tumors were ulcerated (Table 3). According to Lauren's classification 75% of tumors were intestinal, 19.6% diffuse and 5.4% mixed type. The tumors were classified using the Japanese classification system into differentiated (48.2%) and non-differentiated (51.8%). Most of the operated tumors were T3 (44.6%) or T2 (37.5%) (Table 3).

Table 2. Characteristics of the patients from the retrospective study (n=81)

Characteristics	*Patients N (%)*		*p-value*
N stage			
N1	78 (96.3)		
N1,N2	0 (0)		
N2 (skip metastases)	3 (3.7)		
N1, N2, N3	0 (0)		
N3 (skip metastases)	0 (0)		
Tumor location and nodal metastases			0.0001
	N1	*N2*	
Proximal	12 (14.9)	2 (2.5)	
Central	20 (24.7)	1 (1.2)	
Distal	46 (56.7)	0 (0)	
T stage and nodal metastases stage			0.0001
	N1	*N2*	
T1	12 (14.8)	0 (0)	
T2	28 (34.6)	0 (0)	
T3	36 (44.4)	2 (2.5)	
T4	2 (2.5)	1 (1.2)	
Tumor histology and nodal metastases			0.0001
	N1	*N2*	
Intestinal	49 (60.6)	0 (0)	
Infiltrative	18 (22.2)	1 (1.2)	
Signet ring cell	11 (13.6)	2 (2.5)	

Mapping of SLNs was successful in 55 of the 56 patients (98.2%) in the prospective study. The only unsuccessful mapping was due to a combination of a late-stage tumor with lymphatic obstruction and the obesity of the patient. Biopsies of SLNs were analyzed using conventional H&E staining and IHC. Patients were classified into appropriate categories depending on the results from both H&E and IHC (Figure 2). We observed a statistically significant change ($p<0.001$) in disease stage after IHC examination of SLNs.

Discussion

SLN mapping and subsequent biopsy have become standard procedures in the treatment strategy of patients with malignant melanoma and breast cancer, while they are still under evaluation in gastric cancer surgery [17]. Tumor depth of invasion and lymph

Table 3. Demographic and clinical characteristics of the patients from the prospective study

	Patients			*Patients*	
Characteristic	*N*	*%*		*N*	*%*
No. of patients	56	100	Location of gastric tumor		
Gender			Proximal	13	23.2
Female	22	39.7	Central	16	28.6
Male	34	60.3	Distal	27	48.2
Age, years (mean±SD)	63.91±9.37		Volume (cm^3)		
Duration of disease (months)			0-2.75	20	35.7
0-3	11	19.6	2.75-4	10	17.9
3-6	25	44.7	4-6	15	26.8
6-12	13	23.2	>6	11	19.6
>12	7	12.5	Borrmann's type		
Helicobacter pylori			Vegetative	1	1.8
Positive	34	61.7	Ulcerated	29	51.8
Negative	22	38.3	Ulcerovegetative	18	32.1
Symptoms			Infiltrative	8	14.3
Pain and dyspepsia	28	50	T stage		
Weight loss	37	66.1	T1	8	14.3
Vomiting	31	55.4	T2	21	37.5
Bleeding	11	19.6	T3	5	44.6
Gastric ulcer	7	12.5	T4	2	3.6

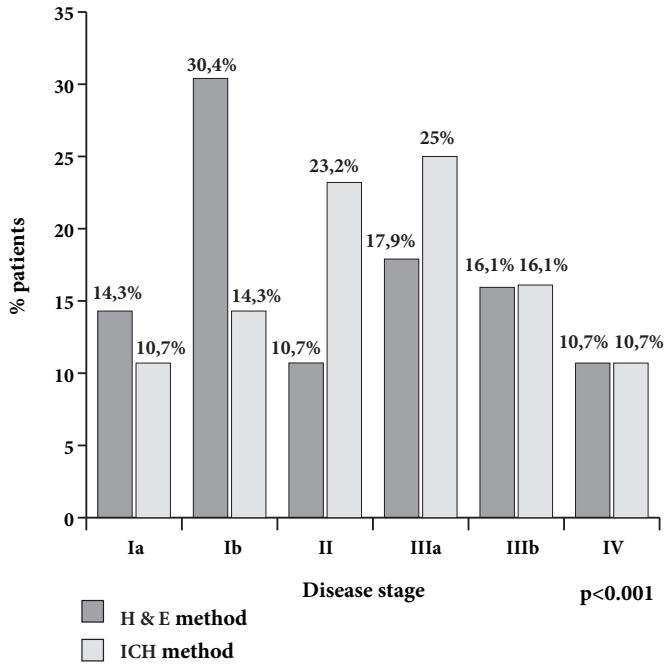

Figure 2. Percent of patients in different disease stages diagnosed by hematoxylin & eosin (H&E) and immunohistochemical (IHC) detection of tumor cells in sentinel lymph node biopsies.

node involvement are two of the most important prognostic factors for patients with gastric carcinoma. Therefore, SLNB is becoming a reliable guide to defining the boundaries of tissues to be resected during oncological surgery [14]. Nevertheless, the extent of lymphadenectomy during therapeutic gastrectomy for gastric cancer is still a controversial issue. Traditionally, extended lymphadenectomy is performed mostly in Eastern Asia, while restricted lymphadenectomy is supported by most Western surgeons [19]. Recent progress in laparoscopic techniques has motivated many gastrointestinal surgeons to look for new methods to easily perform SLNB and to make more accurate decisions as to the extent of lymphatic tissue that should be removed during laparoscopic resection of gastric cancer [14]. Precise detection of SLN could achieve selection of patients not only for minimally invasive curative surgery, but also for the application of a broader spectrum of therapeutic options [20]. The concepts of infrared ray electronic endoscopy, fluorescence imaging, nanoparticles and near-infrared technology are emerging as particularly

promising alternative techniques [14]. On the other hand, H&E staining remains the main method for the detection of SLN metastases, although IHC staining for this type of clinical analysis has been used in several specialized centers [14].

The feasibility, utility and diagnostic reliability of mapping and SLNB in gastric carcinoma have not been fully determined. There are several reasons for this: lymphatic drainage from the gastrointestinal tract is complex; skip metastases are frequently detected in gastrointestinal carcinomas [21]; according to existing models of solitary metastases, their distribution in lymph nodes is quite unpredictable [22]. This explains why some surgeons are quite skeptical towards the application of SLN methodology in gastric cancer [21].

Sano and associates [23] found that lymph nodes in the perigastric region that are located close to the primary tumor represent the first destination for metastases in only 62% of gastric carcinomas. Most other retrospective studies have discussed the question of whether specific regions of the stomach drain to a single node [21]. Kitagawa and Kitajima hypothesized that the SLN of a gastrointestinal tumor is not necessarily located anatomically closest to the primary lesion and is not necessarily the only one, therefore they supposed that the specificity of SLN could be dependent on both the patient and the type of the lesion. Hence, actual data on SNL mapping are more important than retrospective studies on models of tumor metastases [24].

Our study demonstrated a very highly successful SLN mapping and biopsy (98.2%), as well as highest specificity (100%), sensitivity (100%) and accuracy (100%) to predict the presence of metastases in lymph nodes surrounding gastric carcinoma. We determined a highly statistically significant change in the stage of disease after IHC analysis of SLNs ($Z=4.123$; $p<0.0001$) from lower to higher disease stage. In addition, we did not detect skip metastases in our prospective study, which included 56 patients with gastric carcinoma, while we detected a very low incidence of skip metastases (3.7%) in the retrospective study of 82 patients with gastric carcinoma. Skip metastases have been considered the most important limiting factor in the usage of SLNB in gastric carcinoma diagnostics.

We analyzed a total of 137 patients who underwent radical surgery for gastric carcinoma. The research was designed as two independent but correlated studies: retrospective and prospective. The prospective study encompassed a clinical experiment during which we mapped and biopsied SLN in 56 patients in order to determine the feasibility of SLNB as a predictor of lymph node status. Our retrospective study was designed as an analysis of clinical and pathological parameters in 81 patients who had only 1-2 metastases in lymph nodes, in order to determine the frequency of skip metastases in gastric carcinoma.

Our study demonstrated the importance of SNLB in gastric carcinoma diagnostics and treatment [26], but showed also some limitations, with relatively low statistical significance due to the small number of patients. Additional well-designed and multicentre studies including patients with gastric carcinomas at different disease stages are necessary to finally clarify this problem. Using the clinical experiment we confirmed the simplicity and utility of lymphatic mapping and marking which do not harm the patient, but also does not increase treatment costs or increase the hospital stay. The only change we noticed in patients was the color of their urine, which lasted for a few hours post-administration, but without any alteration in biochemical or clinical parameters. We would also like to point out that the T stage (depth of tumor invasion) of our patients was not homogeneous as in some previous studies performed mainly by Japanese authors [7, 26, 27]. Their research was limited only to T1 and T2 tumors, while T2, T3 and T4 tumors were more represented in our patient population.

Because of the cost and waiting time of IHC analysis, we restricted IHC examination of biopsies only to SLNs that were negative for metastases on routine H&E staining. This additional staining with cytokeratin 8/18 antibody allowed us to increase the disease stage in some of the patients. We conclude that this procedure could enable "ultra staging" and additional selection of patients for further cancer treatment. We

also infer that complete IHC analysis of all dissected lymph nodes could give a more precise metastatic status of the lymph nodes.

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